

January 11, 2002

The Honorable Christine Todd Whitman
Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Subject: Comments on DuPont's HPV Test Plan and Robust Summary for the Dinitrile Category

Dear Administrator Whitman:

The following comments on the DuPont test plan for the dinitrile category are submitted on behalf of the Physicians Committee for Responsible Medicine (PCRM), People for the Ethical Treatment of Animals (PETA), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than nine million Americans.

General Comments

DuPont has appropriately formed a category containing three dinitrile compounds. In addition to ethylsuccinonitrile (ESN) and 2-methylglutaronitrile (2-MGN), DuPont has judiciously included adiponitrile (ADN), covered by the OECD SIDS process, to aid in the category analysis. DuPont also presented occupational monitoring data at its two facilities manufacturing 2-MGN. The data indicate that 2-MGN is present at low concentrations in the occupational environment. We commend DuPont's submission of this human exposure data, as this type of information has been glaringly absent from other test plans.

However, DuPont's test plan for the dinitriles calls for mammalian toxicity testing for dermal irritation and eye irritation tests with ESN. A repeat dose test on 2-MGN is also in progress, a violation of the original HPV framework that called for a review of public comments before initiation of any testing. An additional repeat dose test with ESN may be conducted, pending the results of the current repeat dose test of 2-MGN. These tests are inappropriate because a complete HPV SIDS dataset exists for ADN, and this information can be used to extrapolate to the other members of the category. Furthermore, the proposed dermal and eye irritation experiments are beyond the scope of the HPV program and can inflict extreme suffering upon animals. Nonanimal test methods are available and widely used by chemical and pharmaceutical companies the world over.

It is our understanding, based on a letter to PETA from DuPont's Manager of Environmental Stewardship, Edwin L. Mongan III, dated December 11, 2001, and a telephone conversation with PCRM on January 8, 2002, that DuPont will be officially withdrawing the proposals for these irritation tests. We are pleased that

DuPont has recognized that these tests are unnecessary. Given that internationally accepted alternatives exist for these endpoints, it is neither necessary nor appropriate to conduct tests on animals under the HPV program or in any other context.

The original test plan violates the following terms of the October 1999 Agreement among the EPA, industry, and health, animal protection, and environmental organizations, which delineated certain principles for eliminating unnecessary testing on animals:

1. In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach.
3. Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships.
6. Consistent with the OECD/SIDS program, participants generally should not develop any new dermal toxicity data.
10. Companies shall allow 120 days between the posting of test plans and the implementation of any testing plans.

Our main objections to this test plan are as follows:

- A complete HPV SIDS dataset exists for adiponitrile, and, given its close structural and toxicological similarity to the members of the dinitrile category, the available information is sufficient for characterizing the hazard potential of the dinitrile category. The proposed mammalian toxicity tests are inappropriate.
- The category should have been expanded to include other structurally similar HPV chemicals, specifically low-molecular-weight aliphatic nitriles.
- The proposed dermal and eye irritation experiments are beyond the scope of the HPV program and can inflict extreme suffering on animals. Internationally accepted *in vitro* methods are recommended. Therefore, the proposed dermal and eye irritation tests on animals are inappropriate.

SPECIFIC COMMENTS

A complete HPV SIDS dataset exists for adiponitrile, and, given its close structural and toxicological similarity to the members of the dinitrile category, the available information is sufficient for characterizing the hazard potential of the entire dinitrile category. The proposed mammalian toxicity tests are inappropriate.

Nitriles represent a class of chemicals used for manufacturing rubber and nylon, and as intermediates in the synthesis of other organic materials such as hexamethylenediamine and adipoguanamine resins. The three category members are six-carbon straight and branched chain alkanes with nitrile groups on each end. The three chemicals are similar in chemical structure, physical and chemical characteristics, environmental toxicity, aquatic toxicity, and acute toxicity. Because of these similarities, it is reasonable to conclude that category members would exhibit comparable toxicity. Since all SIDS test have been conducted with ADN, these

results can be extrapolated to other chemicals.

In addition, information about other nitrile compounds has been gathered. For example, an August 1991 National Toxicology Program Report states that succinonitrile (CAS #110-61-2), a four-carbon straight chain dinitrile compound is “irritating to skin, eyes, and mucous membranes.”¹ Also, the National Institute of Occupational Safety and Health recommends that concentrations of succinonitrile in air not exceed an eight-hour time-weighted average of 20 mg/m³.

Tetramethyl succinonitrile (CAS #3333526) is another example of a branched dinitrile compound that has been studied. Exposure limits have been set based on its dermal toxicity. The NIOSH REL for skin is 3 mg/m³ or 0.5 ppm, the OSHA PEL is 3 mg/m³ or 0.5 ppm for skin, and the ACGIH TLV is 2.8 mg/m³ or 0.5 ppm for skin.

Exposure to nitriles is already monitored and controlled in DuPont’s workplace. Sufficient information exists to demonstrate the potential toxic properties of these chemicals. Evidence shows that some nitriles are partially metabolized to cyanide, a well-known highly toxic chemical. Exposure to high levels of cyanide harms the brain and heart, and may cause coma and death. Exposure to lower levels may result in breathing difficulties, heart pains, vomiting, blood changes, headaches, and enlargement of the thyroid gland.²

Although DuPont states in its test plan that ADN is not a skin irritant or skin sensitizer according to animal toxicity data, but does cause slight eye irritation in animals, enough information exists to warrant some concern about the potential irritating and corrosive effects of dinitrile chemicals. Even DuPont’s own Material Safety Data Sheet (MSDS) warns that “skin contact with adiponitrile may cause skin irritation with discomfort or rash. Evidence suggests that skin permeation can occur in amounts capable of producing the effects of systemic toxicity. Prolonged exposures by skin contact may lead to skin burns and ulcerations.” The MSDS also states, “Eye contact with Adiponitrile may cause eye irritation with discomfort, tearing, or blurring of vision. Prolonged exposures may lead to eye corrosion with corneal or conjunctival ulceration.”³

Acute toxicity data indicate that all three chemicals exhibit similar acute toxicity. Experiments have been performed via the oral, inhalation, and dermal routes. Mammalian dermal and eye irritation data have been gathered for ADN and 2-MGN. Since data already exist for two members of the category, no further testing is warranted.

The category should have been expanded to include other structurally similar HPV chemicals, specifically low-molecular-weight aliphatic nitriles.

The formation of chemical categories has many advantages. This approach reduces the numbers of animals killed in the HPV program, as well as the time and money spent on testing. Furthermore, it yields greater insight into the relationship between physicochemical properties and toxicity. According to the EPA’s Guidance Document on the Development of Chemical Categories in the HPV Challenge Program, a chemical category is a group of structurally similar chemicals with similar physicochemical and toxicological properties. The structural properties could be common functional groups or common breakdown products.⁴

Other HPV chemicals meet these criteria and therefore should have been incorporated into this category. Some examples of such chemicals include low-molecular-weight aliphatic nitriles such as 2-methylactonitrile (CAS #75865, sponsored under the SIDS program), isobutyronitrile (CAS #75865, sponsored by Eastman Chemical Company), and butanenitrile (CAS #109740, sponsored by Eastman Chemical Company). According to the October 1999 Agreement, chemical companies should coordinate with each other to maximize the use of chemical

categories. The EPA needs to encourage this type of inter-industry communication and cooperation.

The proposed dermal and eye irritation experiments are beyond the scope of the HPV program and can inflict extreme suffering on animals. Internationally accepted *in vitro* methods are recommended. Therefore, the proposed dermal and eye irritation tests on animals are inappropriate.

The proposed dermal and eye irritation experiments are beyond the scope of the HPV program and represent a blatant disregard for animal welfare issues. Although DuPont has publicly stated it will withdraw this aspect of its test plan, we are providing the following information for future reference. First, irritation data has already been collected on two dinitrile chemicals. Given the similarities among chemicals, no additional irritation data should be collected. Second, the tests inflict a great deal of pain and distress on animals. Third, internationally accepted nonanimal methods exist for these two endpoints. The proposed tests are inappropriate.

To show good faith in adherence to the terms and spirit of the HPV program, DuPont should disclose all information on dermal toxicity, irritation, and corrosion, especially since it had called for additional tests for these endpoints. However, DuPont did not present results from three of its dermal irritation studies, claiming that the “focus of the study was skin corrosion.”⁵ Corrosion is, in fact, irreversible irritation. The October 1999 Agreement calls for companies to apply thoughtful toxicology and to be forthcoming with all available data.

Several internationally accepted alternative methods for assessing eye and skin irritancy are available and described in detail below.

Alternative Eye Irritation Tests

Since the eye is more sensitive to chemical damage than the skin, a nonanimal skin corrosion test should precede any assessment of eye irritation. If a substance is classified as corrosive to the skin, it can also be labeled an eye irritant and corrosive without further testing on animals.

Nonanimal tests for eye irritation are used extensively by industry in product development. While they have not generally been accepted by government regulators for use in chemical risk assessments, several of these tests have received provisional acceptance by government regulators in Europe, including the HPT-CAM assay (using fertilized chicken eggs) and the DCOP assay (using isolated corneas from cattle).

In addition to these methods, the German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) has stated that a simple *in vitro* test of protein precipitation could further discriminate between chemicals that are moderate or severe eye irritants. Substantial protein precipitation in a test such as the Irritection system (formerly Eytex) would indicate irreversible eye irritancy.

***EpiOcular*TM**

Another promising method that is currently being reviewed by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is *EpiOcular*TM. Sponsored by the Colgate-Palmolive Company, *EpiOcular*TM is a three-dimensional, *in vitro* tissue model of the human corneal epithelium. The model consists of normal, human-derived epidermal keratinocytes cultured on a permeable polycarbonate membrane. This construct forms a stratified, squamous, multi-layered epithelium similar to that of the cornea. The tissue construct has an air-liquid interface and exhibits morphological and growth characteristics comparable to the viable human eye. Chemicals can be directly applied to the surface of the tissue to predict potential eye-damage in

human. Time to 50-percent cell death (ET50) is the endpoint for comparison among different potential toxicants. The model provides data that reflects how cell cytotoxicity can be measured and related to the assessment of ocular irritation. This test appears to accurately predict the ocular-irritating effects of chemicals and provides an *in vitro* alternative to the Draize test. The issue of replacing the Draize test with a nonanimal test has been an ongoing problem due to the recognized subjectivity and unreliability of the non-validated Draize test.⁶

A new study by Unilever evaluates how well five *in vitro* and *ex vivo* tests identify the irritating properties of various shampoos and conditioners. The study showed the correlation between *in vitro* and *in vivo* eye irritation data for the five tests.⁷ Three tests appear to be suitable methods for assessing eye irritation, including EpiOcular™, the isolated rabbit eye method, and the fluorescein leakage assay.

Other *ex vivo* tests currently being reviewed by the European Centre for the Validation of Alternative Methods (ECVAM) include the isolated rabbit eye, the bovine cornea opacity and permeability test, and the isolated chicken eye test.

Alternative Dermal Irritation Methods

Corrosive substances are defined as chemicals that cause visible destruction of, or irreversible alterations in, living tissue by chemical action at the site of contact. Dermal corrosivity testing is conducted to identify chemicals that potentially pose this hazard to humans. For regulatory purposes, the *in vivo* test method used often involves applying chemicals on the skin of a rabbit. The chemical is typically allowed to sit on the skin of the live animal for approximately 24 hours, while observations of the skin are made. Concerns about the ethics and scientific value of this test have prompted scientists to develop alternative methods that do not rely on the use of live animals.

Corrositex®

Corrositex® is an *in vitro* method used to determine the dermal corrosive potential of chemicals. It is based on the ability of a corrosive chemical to penetrate or destroy a biobarrier matrix and to elicit a color change in an underlying Chemical Detection System. The ICCVAM Corrosivity Working Group found that Corrositex® is useful as a replacement assay for evaluating the corrosivity or noncorrosivity of acids, bases, and acid derivatives, and that the method showed good specificity, sensitivity, and accuracy.⁸ In its letter to PETA, DuPont acknowledges that it “has been using Corrositex® for more than five years.”

Transcutaneous Electrical Resistance (TER) Test

The rat skin Transcutaneous Electrical Resistance (TER) test involves the application of chemicals to skin taken from dead rats. Corrosive materials are identified by the ability to produce a loss of normal stratum corneum integrity and barrier function, which is measured as a reduction of the inherent transcutaneous electrical resistance. Although this method clearly involves the use of animals, it represents a step in the right direction, as it does not apply potentially corrosive chemicals to the shaved skin of live animals.

In March 1998, ECVAM unanimously concluded that the rat skin TER test is scientifically valid for use as a replacement for the animal test for distinguishing between corrosive and noncorrosive substances, and that the test be considered for regulatory acceptance. The results of the ECVAM international validation study on *in vitro* tests for skin corrosivity were reproducible within and among the three laboratories that performed the test. The rat skin TER test proved to be applicable to a diverse group of 60 chemicals, including organic acids,

bases, neutrals, inorganic salts, electrophiles, phenols, and soaps/surfactants. The skin corrosivity *in vitro* data correlated well with the *in vivo* data.⁹

Episkin™

The Episkin™ assay uses a human-reconstructed epidermis and a functional stratum corneum to determine the dermal corrosivity properties of chemicals. The chemical is topically applied to the surface of the skin for 3, 60, and 240 minutes, with subsequent assessment of its effects on cell viability. In March 1998, ECVAM unanimously agreed that the Episkin™ test is scientifically valid for use as a replacement method for the animal test, and that it is ready to be considered for regulatory acceptance. The validation study involved 3 laboratories and 60 chemicals. Correlation between *in vitro* and *in vivo* data was high. The test was able to correctly identify corrosive and noncorrosive chemicals.¹⁰

EpiDerm™

The EpiDerm™ Skin Model is a nonanimal, *in vitro* method for assessing dermal irritancy and toxicology. EpiDerm™ is composed of normal, human-derived epidermal keratinocytes that have been cultured to form a multi-layered, highly differentiated model of the human epidermis. EpiDerm™ closely models the barrier function properties of human skin.¹¹

The rat skin TER test, EpiSkin™, and EpiDerm™ are all currently under review by ICCVAM.

Nonanimal tests for skin irritancy also exist and are used extensively by industry in product development. If a chemical is classified as a corrosive on the basis of one of the validated animal tests described above, it should also be classified as an irritation without further testing (since skin corrosion is simply an irreversible form of skin irritation). Alternatively, the ability of noncorrosive chemicals to cause skin irritation may be assessed using minimally-invasive skin patch tests on human volunteers, if deemed necessary and appropriate. Other companies, such as Procter and Gamble, support using these *in vitro* tests as stand-alone tests.¹²

Given that alternative tests have been fully validated, having satisfied all of the internationally accepted criteria for their reliability and relevance for their intended purpose, it is neither necessary nor appropriate to conduct animal tests. However, the test plan could be further improved and testing further reduced by expanding the category to include other low-molecular-weight aliphatic nitriles and by including additional, already available information about the potential hazards of these chemicals.

Thank you for your attention to these comments. I can be reached at 202-686-2210, ext. 302, or via e-mail at ncardello@pcrm.org.

Sincerely,

Nicole Cardello, M.H.S.
Staff Scientist

References

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